Z. Säugetierkunde 55 (1990) 99–105

ISSN 0044-3468

Cytogenetics of Vesper mice, *Calomys* (Rodentia; Cricetidae): Robertsonian variation between *Calomys callidus* and *Calomys venustus*

By A. D. Vitullo, María B. Espinosa and María S. Merani

Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Receipt of Ms. 3. 8. 1988

Abstract

Investigated karyotype relationships between *Calomys callidus* and *Calomys venustus* to determine the chromosomal repatternings involved in karyological differentiation. Chromosomal analysis was performed in seven specimens of *C. venustus* from San Luis and Córdoba provinces, Argentina. A 2n = 56 (NF = 69) karyotype similar to that previously reported for specimens from the type locality was found. G-band comparisons showed that *C. venustus* and *C. callidus* (2n = 48, NF = 69) karyotypes were interrelated by means of four Robertsonian rearrangements which accounted both for numerical and structural changes. These data, together with those previously reported for other species of the genus, support the existence of three karyological groups within *Calomys*: group I, karyotypes with higher chromosomal numbers (2n = 64–60), mostly telocentric; group II, asymmetrical karyotypes (2n = 56–48) interrelated by fusion-fission processes; and group III, karyotypes with lower diploid numbers (2n = 38–36) representing highly rearranged states from an all-telocentric ancestral condition (2n = 70).

Introduction

Systematics of the South American genus *Calomys* (Cricetidae, Sigmodontinae) is still not completely clear. Both the number of species and their relationships are poorly understood, as reflected in the variability of classifications recognized by different authors (Thomas 1916; Tate 1932; Ellerman 1941; Cabrera 1961; Hershkovitz 1962; Honacki et al. 1982; Reig 1984a). Recently, however, cytogenetic data have been accumulated, proving a useful tool to clarify species distinction in these rodents (Reig 1984b; Vitullo et al. 1984).

It is generally assumed that the species belonging to the genus *Calomys*, on the basis of their larger or smaller size, are to be classified in two distinct groups. Nevertheless, there are no conclusive studies to elucidate whether this distinction is a morphological convergence or a cladistic divergence (Corti et al. 1987). Hershkovitz (1962) has proposed that all the large *Calomys* with sharp interorbital edges belong to the same species, including them under the name *C. callosus* (Rengger 1830). The cytogenetic study of specimens referred to as *C. callosus* in Argentina, according to Hershkovitz' classification, has shown gross chromosomal differences to those from the type locality of *C. callosus* in Paraguay (Hurtado de Catalfo and Wainberg 1974; Lisanti et al. 1976; Pearson and Patton 1976; Gardenal et al. 1977). In the light of these differences, the revalidation of *C. venustus* (Thomas 1894) was proposed for the large *Calomys* distributed in Argentina (Forcone et al. 1980; Reig 1984b).

In this paper we analyse the chromosomal relationships between *C. venustus* and *C. callidus*, showing that both species together with *C. fecundus* from Bolivia make up a distinctive group of species within the genus, according to their karyotype features. In addition, cytogenetics is reviewed in order to evaluate the karyotype evolution of the genus *Calomys*.

Material and methods

Chromosomal analysis was performed in 7 *C. venustus* from Donovan, San Luis province (4 males), and Cosquín, Córdoba province (1 male, 2 females) from Argentina. Voucher specimens were preserved at the Museo Municipal de Ciencias Naturales Lorenzo Scaglia, Mar del Plata, Argentina.

Chromosomes were obtained from bone marrow. Briefly, the animals were injected with colchicine (1 μg/g body weight), and killed 2 to 3 hours later by an overdose of ether anesthesia. Hypotonic treatment was performed in 0.075 M KCl for 20 min at 37 °C. Cells were fixed in cold 3:1 methanol:acetic acid. Chromosomal spreads were air-dried and Giemsa stained. G-bands were induced by Trypsin digestion according to Seabright (1971). Chromosomes were classified according to Levan et al. (1964), and arranged and numbered by decreasing size. Fundamental numbers are referred to as NF, autosomal arms plus XY arms, or NF_a, autosomal arms only.

G-banded karyotypes of both *C. venustus* and *C. callidus* were arm-to-arm compared, and chromosomal similarities were established by equivalent banding patterns and sizes. The data corresponding to *C. callidus* were reanalysed from the material reported in a previous paper (Vitullo et al. 1984).

Interspecific crosses between *C. callidus* females and *C. venustus* males were established in monogamous pairs under laboratory conditions previously standardized for *C. callidus* (Hodara et al. 1984; Merani et al. 1988).

Results and discussion

The 7 specimens of *C. venustus* analysed showed an identical 2n = 56 (NF = 69) karyotype (Fig. 1). The first five pairs of autosomes were metacentric chromosomes decreasing in size. The remaining autosomal pairs were made up by telocentric chromosomes grading in size, with the exception of pair 19 which comprised small metacentric chromosomes. Sex chromosomes were XY with a submetacentric X, and a small subtelocentric Y chromosome (Table 1). This karyotype was similar to a previously reported one from the type locality, Cosquín (Gardenal et al. 1977). No differences in diploid number, chromosome morphology, and G-banding pattern were found in specimens from San Luis province.

C. callidus showed a 2n = 48 (NF = 69) karyotype with nine large distinctive metacentric pairs (Fig. 1) (Vitullo et al. 1984). Arm-to-arm comparisons of G-banded karyotypes showed identical *C. venustus* and *C. callidus* chromosomal complements, as well as the existence of four Robertsonian repatternings which accounted both for numerical and structural changes (Fig. 2).

Back in 1894, specimens from Cosquín, Córdoba were named *C. venustus* by Thomas. Cytogenetic studies on these animals from Córdoba and Tucumán provinces have shown 2n = 56 and 2n = 54 karyotypes, respectively (Hurtado de Catalfo and Wainberg 1974; Lisanti et al. 1976; Gardenal et al. 1977). However, in both reports the animals were referred to as *C. callosus* (Rengger, 1830) according to Hershkovitz (1962) who considered *C. venustus* as a synonym of *C. callosus*. Forcone et al. (1980) and Reig (1984b) have proposed the revalidation of *C. venustus* as a full species in the light of the finding of a 2n = 36 karyotype in specimens of *C. callosus* from the type locality, Paraguay (Pearson and Patton 1976). Furthermore, Thomas (1916) distinguished two subspecies of *C. venustus*: *C. v. venustus* distributed from Córdoba province to the northwest Argentina, and *C. v. callidus* restricted to the Mesopotamian region. Cytogenetic studies in *C. v. callidus* showed a 2n = 48 karyotype (Vitullo et al. 1984). It was then proposed that chromosomal variation was so striking that it must be taken as an indication of the establishment of a natural reproductive barrier; thus, it was suggested that *C. callidus* must be considered as a full species (Vitullo et al. 1984). 4 out of 9 interspecific crosses between *C. callidus* females and *C. venustus* males delivered at least once. F_1 hybrids showed a 2n = 52 (NF = 69) karyotype with 4 heteromorphic pairs. Although interspecific gestation proceeded to term, it was found that the F_1-males were sterile (Vitullo, unpubl. results). This reinforces our early hypothesis that *C. callidus* is reproductively isolated from *C. venustus* (Vitullo et al. 1984).

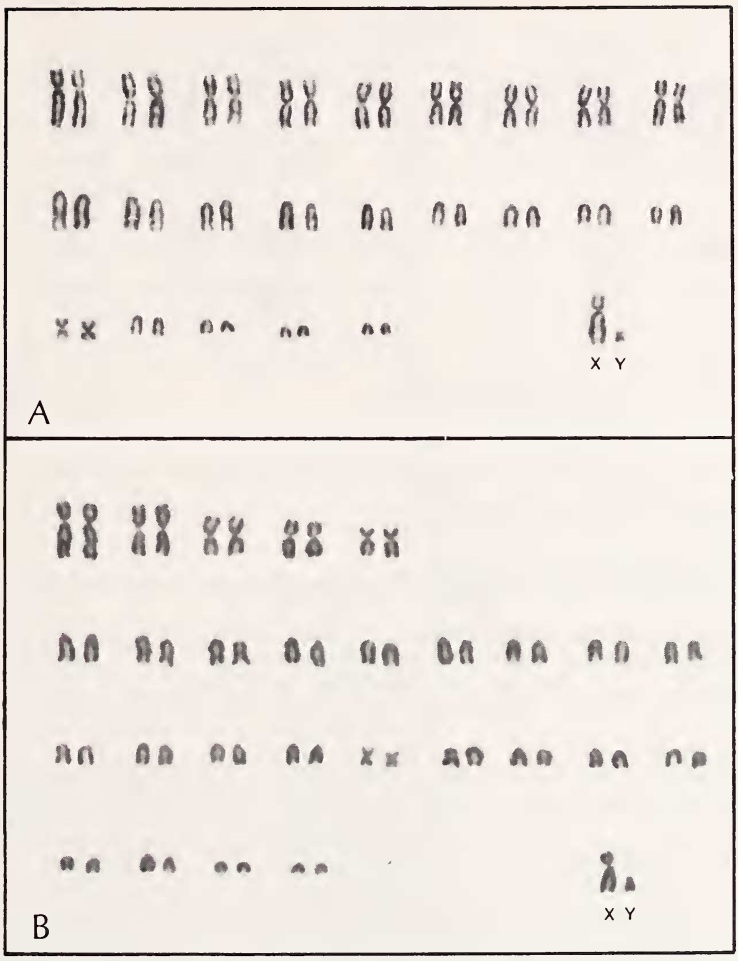

Fig. 1. Giemsa-stained karyotypes of: A – *Calomys callidus* (2n = 48, NF = 69); B – *Calomys venustus* (2n = 56, NF = 69)

Hershkovitz (1962) has argued that all the large *Calomys* with sharp interorbital edges belong to one species, namely *C. callosus*. According to this criterion, *C. venustus* and *C. callidus* from Argentina, and *C. fecundus* from Bolivia were synonymized with *C. callosus*. However, chromosomal data here presented suggest that species distinction made by Thomas is more consistent than Hershkovitz' reclassification.

Pearson and Patton (1976) have proposed that *Calomys* species can be divided into two distinctive groups, *C. laucha-C. sorellus* and *C. lepidus-C. musculinus-C. callosus*, according to their chromosomal relationships. In addition, these authors proposed a somewhat intermediate position for *C. fecundus* between the above mentioned groups, to which *C. venustus* and *C. callidus* could be added. These three species show intermediate diploid numbers at the range displayed by the genus (2n = 64 to 2n = 36), forming a Robertsonian series from 2n = 56 to 2n = 48. Although banding patterns are not available for *C. fecundus*, its karyotype (2n = 50, NF = 69 with eight large distinctive biarmed autosomes) (Pearson and Patton 1976) and chromosomal lengths support the inclusion

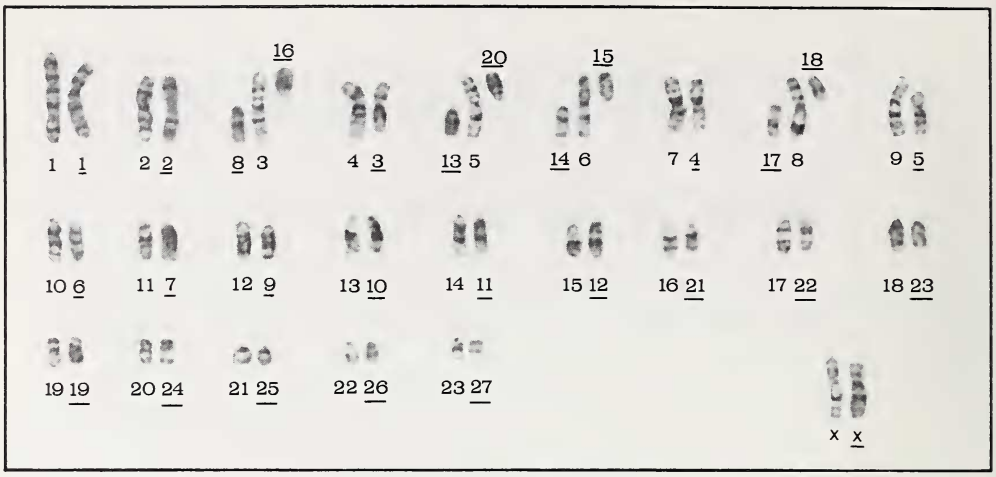

Fig. 2. Comparison of G-banded karyotypes from *Calomys callidus* and *Calomys venustus*. Underlined numbers indicate the *C. venustus* haploid set

Table 1. **Chromosomal lengths of *Calomys callidus* and *Calomys venustus* expressed as percentage of the female haploid set**

Chromosome	*C. callidus* X ± SD	*C. venustus* X ± SD
1	7.24 ± 0.62 (1.61)*	7.70 ± 0.45 (1.64)
2	6.47 ± 0.46 (1.17)	7.36 ± 0.36 (1.00)
3	6.32 ± 0.36 (1.24)	6.00 ± 0.52 (1.09)
4	6.03 ± 0.40 (1.25)	5.37 ± 0.41 (1.16)
5	5.80 ± 0.26 (1.23)	4.36 ± 0.53 (1.43)
6	5.61 ± 0.28 (1.20)	4.10 ± 0.19
7	5.30 ± 0.20 (1.19)	3.89 ± 0.28
8	5.04 ± 0.38 (1.25)	3.59 ± 0.17
9	4.72 ± 0.31 (1.29)	3.55 ± 0.16
10	4.17 ± 0.28	3.47 ± 0.23
11	3.91 ± 0.22	3.38 ± 0.33
12	3.73 ± 0.22	3.26 ± 0.32
13	3.54 ± 0.24	3.09 ± 0.20
14	3.36 ± 0.30	3.00 ± 0.22
15	3.21 ± 0.25	2.83 ± 0.11
16	3.02 ± 0.26	2.83 ± 0.11
17	2.88 ± 0.22	2.83 ± 0.12
18	2.75 ± 0.19	2.70 ± 0.21
19	2.66 ± 0.28 (1.09)	2.73 ± 0.35 (1.19)
20	2.52 ± 0.26	2.62 ± 0.27
21	1.92 ± 0.40	2.54 ± 0.25
22	1.72 ± 0.30	2.45 ± 0.22
23	1.52 ± 0.36	2.41 ± 0.22
24		2.37 ± 0.17
25		2.16 ± 0.15
26		1.77 ± 0.26
27		1.56 ± 0.14
X	6.64 ± 1.27 (1.81)	6.02 ± 0.43 (1.75)
Y	2.26 ± 0.29	2.29 ± 0.55

* Numbers in parenthesis indicate the centromeric index (long arm/small arm) of biarmed chromosomes.

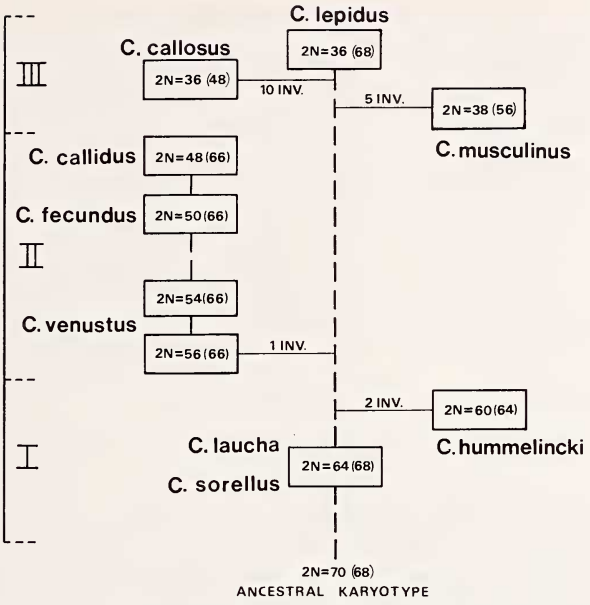

Fig. 3. Interpreted pathway of chromosomal changes within *Calomys*. Each vertical line indicates a Robertsonian fusion, and horizontal lines indicate superimposed pericentric inversions. Number in paranthesis are NF_a. Roman numbers refer to species grouping according to karyotype features and the degree of transformation from the ancestral condition (see text)

of this species in the Robertsonian series. In support, *C. venustus, C. callidus* and *C. fecundus* are more closely related to one another than *C. callosus* which shows a 2n = 36 (NF_a = 48) karyotype with 7 pairs of large biarmed chromosomes, a small-sized metacentric, and 10 pairs of subtelocentrics and telocentrics (Pearson and Patton 1976), suggesting that other rearrangements are superimposed on Robertsonian ones.

A basic pattern of chromosomal evolution by decrease in diploid numbers through Robertsonian reduction has been independently proposed for several major Neotropical Cricetidae: Sigmodontini (Zimmerman 1970), Akodontini (Bianchi et al. 1971; Vitullo et al. 1986), Oryzomyini (Gardner and Patton 1976), and Phyllotini (Pearson and Patton 1976). A karyotype of 68 telocentric autosomes (2n = 70, NF_a = 68) has been interpreted as the ancestral condition for the genus *Phyllotis* and the phyllotini group in general. This all-telocentric karyotype appears to have given rise to those represented in *Calomys* species through a basic series of 17 fusions leading to the all-metacentric karyotype (2n = 36, NF_a = 68) displayed by *C. lepidus* (Fig. 3). Throughout such a basic trend in chromosomal evolution, *Calomys* species may have developed in progressive steps involving a minimum of chromosome changes. Thus, it is possible to discern three groups of species in *Calomys* according to karyotype features (Table 2).

Group I includes karyotypes with higher chromosomal numbers (2n = 64–60) within the genus, closely related to the ancestral condition (2n = 70). These karyotypes, mostly telocentric, may be derived from a few Robertsonian repatternings. *C. laucha* from Argentina, *C. sorellus* from Perú, and *C. hummelincki* from Venezuela belong to this group (Fig. 3).

Group II comprises karyotypes ranging from 2n = 56 to 2n = 48 and forming a Robertsonian series at an intermediate position in the basic pathway of transformation from the ancestral type (Fig. 3). These karyotypes are clearly asymmetrical showing a

Table 2. **Grouping of *Calomys* species according to karyotype features**

Karyological Group	Species	2n	NF_a*	Reference
I	*C. laucha*	64	68	Gardenal et al. 1977
	C. sorellus	64	68	Pearson and Patton 1976
	C. hummelincki	60	64	Pérez-Zapata et al. 1987
II	*C. venustus*	56	66	Lisanti et al. 1976
		54	66	Hurtado and Wainberg 1974
	C. fecundus	50	66	Pearson and Patton 1976
	C. callidus	48	66	Vitullo et al. 1984
III	*C. musculinus*	38	56	Forcone et al. 1980
	C. callosus	36	48	Pearson and Patton 1976
	C. lepidus	36	68	Pearson and Patton 1976

* NF_a: number of autosomal arms.

variable number of large distinctive biarmed chromosomes. *C. venustus* and *C. callidus* from Argentina, and *C. fecundus* from Bolivia are included here.

Group III shows a greater heterogeneity when compared with the other two groups, as reflected in NF_a variability (Fig. 3). It contains karyotypes quite distant from the ancestral condition, which display a high degree of transformation by means of pericentric inversions. These karyotypes are mainly made up by metacentric, submetacentric and subtelocentric chromosomes. Except for *C. lepidus* which exhibits an all-metacentric karyotype, the other species included in this group, i.e. *C. musculinus* and *C. callosus*, show a variable number of subtelocentric chromosomes requiring up to 10 inversions superimposed on Robertsonian repatternings, to explain their appearance (Fig. 3).

The working hypothesis developed here on the basis of available chromosomal data may find support in a progressive knowledge of other species of *Calomys*, especially those distributed in Bolivia and Brazil, for which cytogenetics is still incipient.

Acknowledgements

The authors wich to thank Dr. Cristina Gardenal and Dr. Alfredo Castro-Vázquez for providing the specimens from Córdoba and San Luis, respectively. Thanks also to Dr. J. Blejer and Dr. L. Martínez-Peralta for their help in preparing the German summary. This work was supported by grants from SECYT, and CONICET (by-product of PID-3907102/85), Argentina.

Zusammenfassung

Cytogenetik an Vespermäusen, Calomys (Rodentia, Cricetidae): Robertson'sche Variation zwischen Calomys callidus und Calomys venustus

Die Beziehungen zwischen Karyotypen von *Calomys callidus* und *Calomys venustus* wurden untersucht, um weitere Daten zur karyologischen Differenzierung in diesem Genus zu gewinnen. 7 Individuen von *C. venustus* aus den argentinischen Provinzen San Luis und Córdoba zeigten den gleichen Karyotypen (2n = 56, NF = 69) wie vorher für Tiere der Typenlokalität beschrieben. Vergleiche von G-Bandenmustern ergaben ferner, daß die Karyotypen von *C. venustus* und *C. callidus* durch 4 Robertson'sche Abstände voneinander getrennt sind. Diese und bereits bekannte Daten von anderen *Calomys*-Arten führen zu einer Annahme von folgenden 3 karyologischen Gruppen innerhalb des Genus: Gruppe I: Karyotypen mit größeren Chromosomenzahlen (2n = 64–60), zumeist telozentrisch; Gruppe II: asymmetrische Karyotypen (2n = 56–48), durch Fusionen und Fissionen entstanden; Gruppe III: Karyotypen mit geringen Chromosomenzahlen (2n = 38–36). Diese repräsentieren stark reorganisierte Stadien, die von einem telocentrischen ancestralen Zustand mit hoher Anzahl (2n = 70) entstanden sein mögen.

References

Bianchi, N. O.; Reig, O. A.; Molina, O. J.; Dulout, F. N. (1971): Cytogenetics of the South American akodont rodents (Cricetidae). I. A progress report of Argentinian and Venezuelan forms. Evolution **25,** 724–736.

Cabrera, A. (1961): Catálogo de los mamíferos de América del Sud. Rev. Mus. Arg. Cienc. Nat., Zool. **4,** 309–732.

Corti, M.; Merani, M. S.; de Villafañe, G. (1987): Multivariate morphometrics of Vesper mice *(Calomys)*: preliminary assessment of species, population and strain divergence. Z. Säugetierkunde **52,** 236–242.

Ellerman, J. R. (1941): The families and genera of living rodents. British Museum (Nat. Hist.) **2,** 1–690.

Forcone, A. E.; Luna, M. V.; Kravetz, F. O.; Lisanti, J. A. (1980): Bandas C y G de *Calomys musculinus* (Rodentia, Cricetidae). Mendeliana **4,** 57–65.

Gardenal, C. N.; Triay de Juárez, N.; Gutiérrez, M.; Sabattini, M. S. (1977): Contribución al conocimiento de tres especies del género *Calomys* (Rodentia, Cricetidae). I. Estudios citogenéticos. Physis, secc. C **36,** 169–178.

Gardner, A.; Patton, J. (1976): Karyotypic variation in oryzomic rodents (Cricetinae) with comments on chromosomal evolution in the neotropical cricetine complex. Occas. Papers Mus. Zool. Louisiana State Univ. **49,** 1–48.

Hershkovitz, P. (1962): Evolution of Neotropical cricetine rodents (Muridae) with special reference to the phyllotine group. Fieldiana Zool. **46,** 1–524.

Hodara, V. L.; Kajon, A. E.; Quintans, C.; Montoro, L.; Merani, M. S. (1984): Parámetros métricos y reproductivos de *Calomys musculinus* (Thomas, 1913) y *Calomys callidus* Thomas, 1916 (Rodentia, Cricetidae). Rev. Mus. Arg. Cienc. Nat., Zool. **13,** 453–459.

Honacki, J. H.; Kinman, K. E.; Koeppl, J. W. (1982): Mammal species of the world: a taxonomic and geographical reference. Lawrence, Ka.: Allen Press and Ass. Systematic Coll.

Hurtado de Catalfo, G. E.; Wainberg, R. L. (1974): Citogenética de *Calomys callosus* (Rengger, 1830) (Rodentia, Cricetidae). Análisis métrico del cariotipo somático. Physis, secc. C **33,** 215–219.

Levan, A.; Fredga, C.; Sandberg, A. A. (1964): Nomenclature for the centromeric position on chromosomes. Hereditas **52,** 201–220.

Lisanti, J. A.; Kravetz, F. O.; Ramírez, C. L. (1976): Los cromosomas de *Calomys callosus* (Rengger) (Rodentia, Cricetidae) de la provincia de Córdoba. Physis, secc. C **35,** 221–230.

Merani, M. S.; Hodara, V. L.; Roldán, E. R. S.; Quintans, C. (1988): Reproductive aspects of *Calomys musculinus* and *Calomys callidus* (Rodentia, Cricetidae) under laboratory conditions. Lab. Anim. (in press).

Pearson, O. P.; Patton, J. L. (1976): Relationships among South American phyllotine rodents based on chromosome analysis. J. Mammalogy **57,** 339–350.

Pérez-Zapata, A.; Vitullo, A. D.; Reig, O. A. (1987): Karyotypic and sperm distinction of *Calomys hummelincki* from *Calomys laucha* (Rodentia, Cricetidae). Acta Cient. Venez. **38,** 90–93.

Reig, O. A. (1984a): Distribução geográfica e história evolutiva dos reodores muroideos Sulamericanos (Cricetidae: Sigmodontinae). Rev. Brasil. Genet. **7,** 333–365.

— (1984b): Significado de los métodos citogenéticos para la distinción e interpretación de las especies, con especial referencia a los mamíferos. Rev. Mus. Arg. Cienc. Nat., Zool. **13,** 19–94.

Seabright, M. A. (1971): A rapid banding technique for human chromosomes. Lancet **2,** 971–972.

Tate, G. H. H. (1932): The taxonomic history of certain South and Central American cricetid rodents: *Neotomys* with remarks upon its relationships; the cotton rats (*Sigmodon* and *Sygmomys*), and the fish-eating rats (*Ichthyomys, Anetomys, Rheomys, Neusticomys* and *Daptomys*). Am. Mus. Novitates **583,** 1–10.

Thomas, O. (1916): On the grouping of the South American Muridae that have been referred to *Phyllotis, Euneomys,* and *Eligmodontia*. Ann. Mag. Nat. Hist., ser. 8, **17,** 139–143.

Vitullo, A. D.; Kajon, A. E.; Percich, R.; Zuleta, G.; Merani, M. S.; Kravetz, F. O. (1984): Caracterización citogenética de tres especies de roedores (Rodentia, Cricetidae) de la República Argentina. Rev. Mus. Arg. Cienc. Mat., Zool. **13,** 491–498.

Vitullo, A. D.; Merani, M. S.; Reig, O. A.; Kajon, A. E.; Scaglia, O.; Espinosa, M. B.; Pérez-Zapata, A. (1986): Cytogenetics of South American akodont rodents (Cricetidae): New karyotypes and chromosomal banding patterns of Argentinian and Uruguayan forms. J. Mammalogy **67,** 69–80.

Zimmerman, E. G. (1970): Karyology, systematics and chromosomal evolution in the rodent genus, *Sigmodon*. Publ. Mus. Michigan State Univ., Biol. Ser. **4,** 385–454.

Authors' addresses: A. D. Vitullo, María B. Espinosa, and María S. Merani, Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, piso 11, 1121-Buenos Aires, Argentina